SUPPLEMENTARY INFORMATION

Repurposing endogenous Type I CRISPR-Cas systems for programmable gene repression

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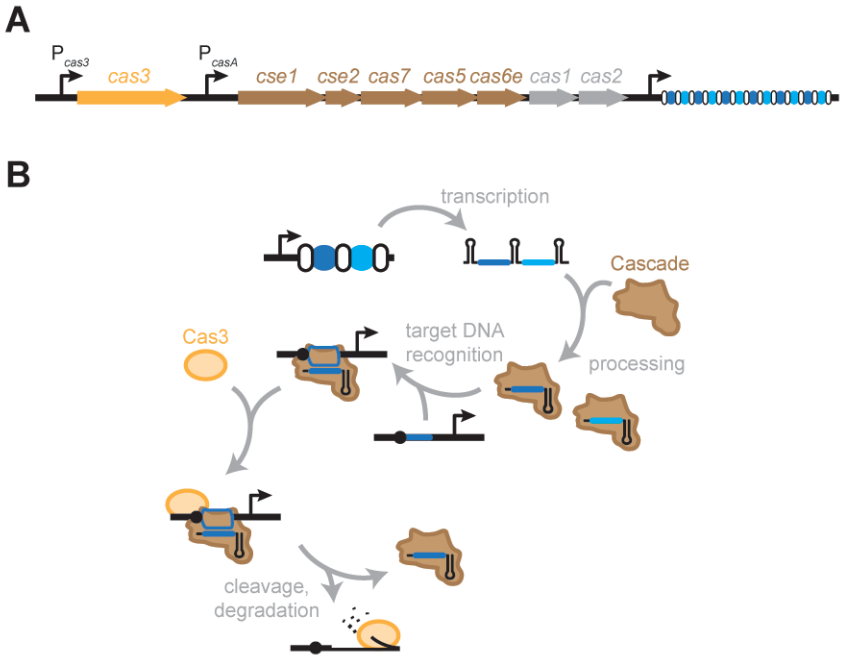
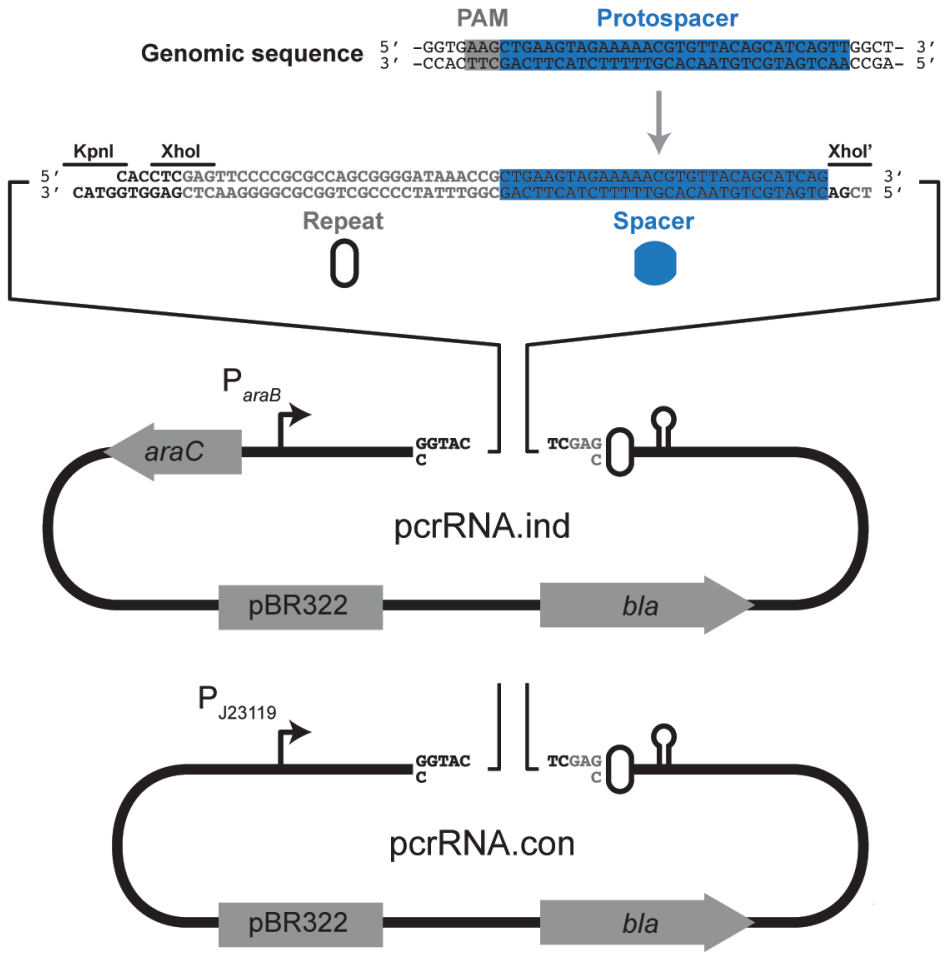
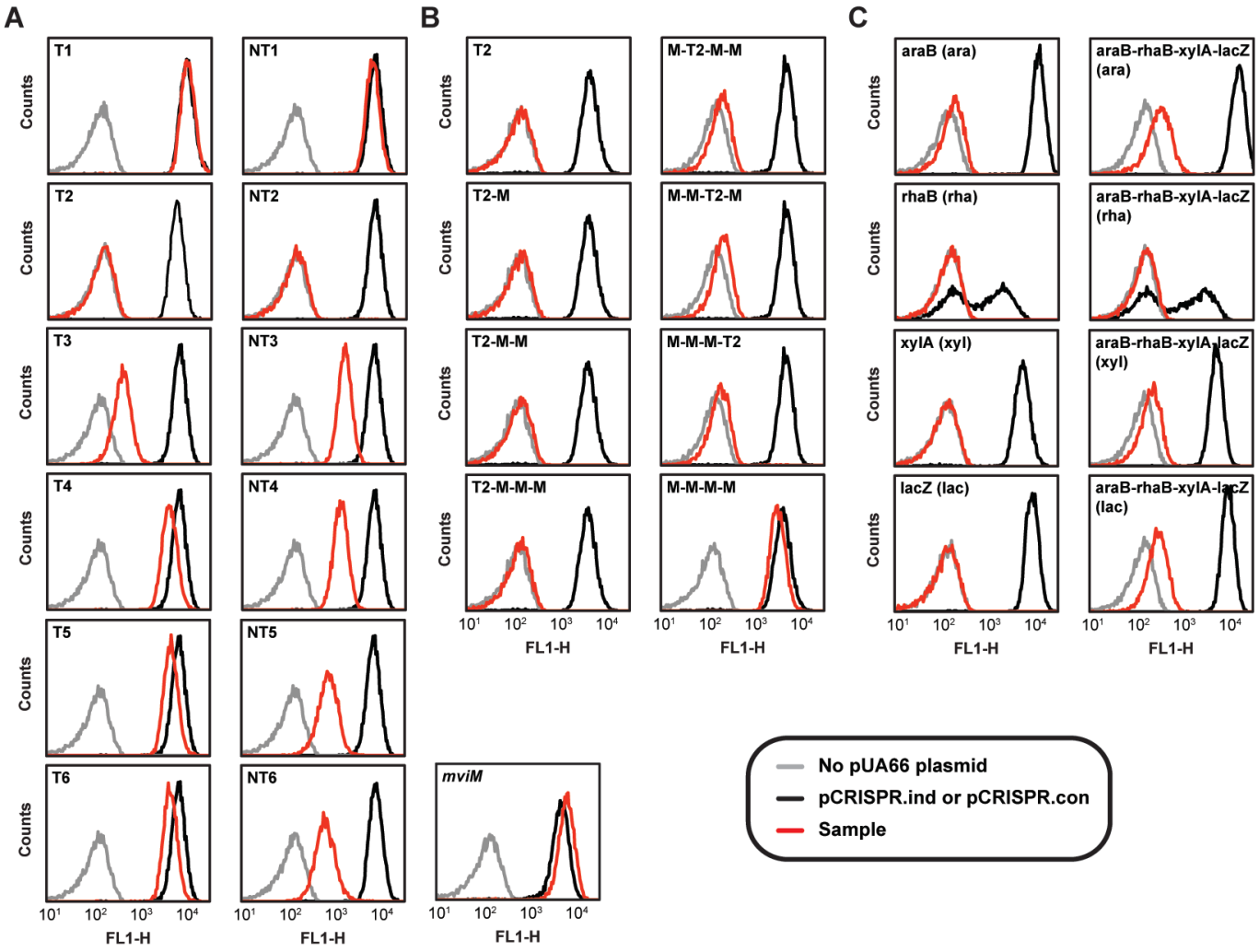
SUPPLEMENTARY FIGURES



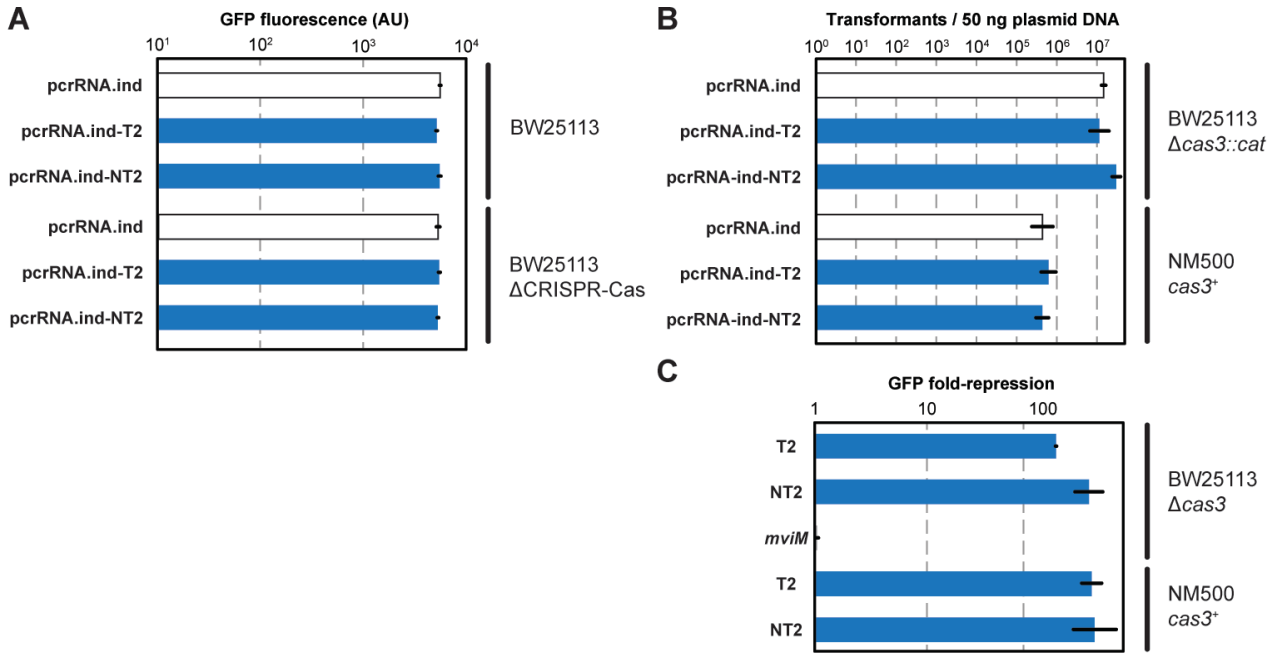
Supplementary Figure S1. The Type I-E CRISPR-Cas system in *E. coli* K-12. (**A**) Genetic locus of the Type I-E system. The *cas3* gene is located upstream of the *cse1-cse2-cas7-cas5-cas6e* operon encoding the Cascade protein complex. The two downstream genes *cas1* and *cas2* are involved in spacer acquisition. The native spacer array is composed of identical repeats (white ovals) and intervening spacers (blue circles). (**B**) Mechanism of DNA destruction based on previous work (1, 2). The transcribed array is processed into individual crRNAs by Cascade. The spacer portion of the array is then used to identify complementary DNA sequences flanked by a PAM (black circle). DNA binding leads to recruitment of Cas3, which cleaves and degrades the target DNA.



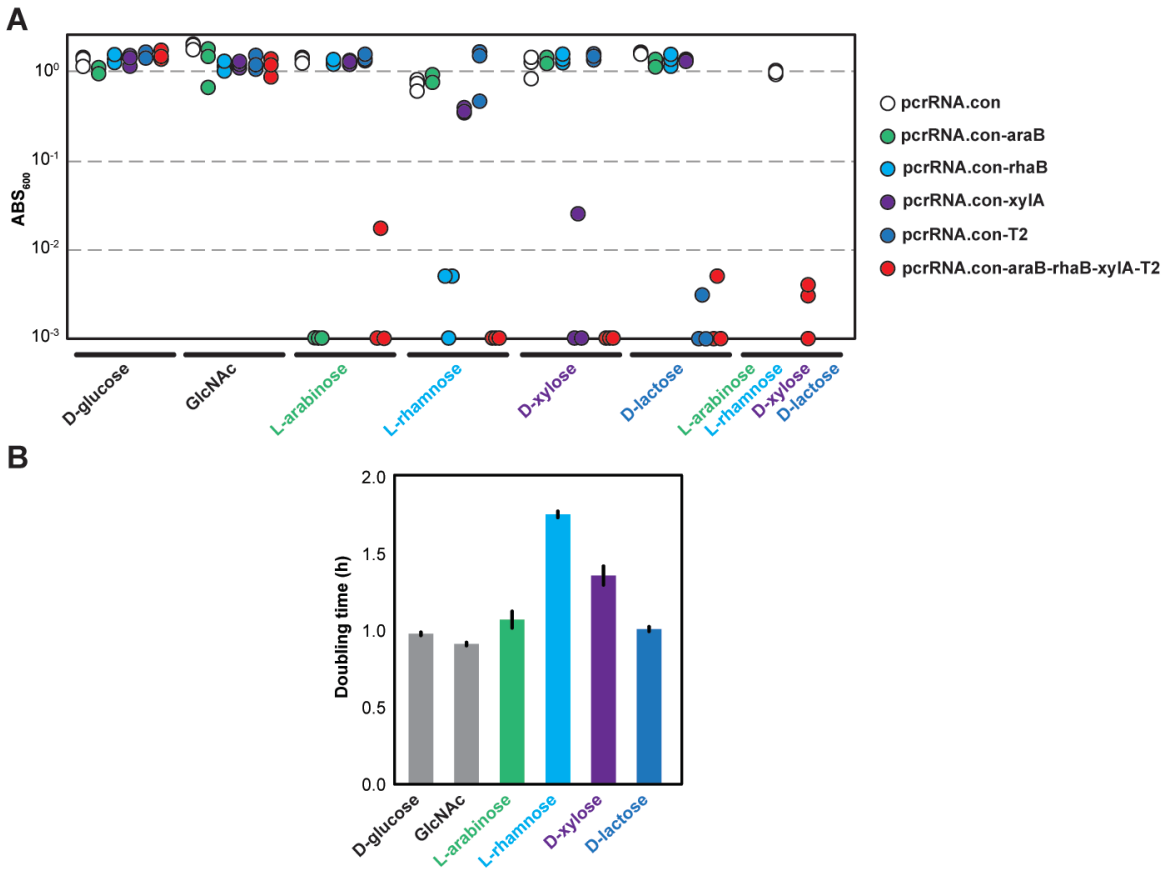
Supplementary Figure S2. Cloning scheme for the synthetic Type I-E CRISPR arrays. Following identification of a PAM in the target sequence, the downstream 32 base pairs serve as the protospacer. All but the last two base pairs are copied into two annealed oligonucleotides that, when annealed, form a repeat-spacer pair. The spacer-repeat pair contains the overhangs for a cleaved KpnI restriction site (left) and cleaved XhoI restriction site (right) along with an internal XhoI restriction site. As long as the 30th base in the protospacer is not a C, ligation of the annealed oligonucleotides into either plasmid digested with KpnI/XhoI disrupts the original XhoI restriction site. Consequently, additional repeat-spacer pairs can be sequentially inserted into the KpnI/XhoI restriction sites.



Supplementary Figure S3. Representative histograms from the flow cytometry analysis. (**A**) Histograms from Figure 2B. (**B**) Histograms from Figure 3. (**C**) Histograms for Figure 4B. The gray histograms are the no-GFP control for autofluorescence, the black histograms are the no-spacer plasmids (either pCRISPR.ind or pCRISPR.con), and the red histograms are the indicated single-spacer or multi-spacer plasmids. See the corresponding figure legend in the main text for more information. Sugars in parentheses were included in the growth medium. Note that the bimodal response to L-rhamnose was reported previously (3). Histograms are representative of independent experiments starting with three separate colonies.



Supplementary Figure S4. GFP expression and DNA transformation in variants of the parent strain BW25113 or NM500. (**A**) GFP fluorescence of BW25113 (top) or BW25113 ΔCRISPR-Cas (bottom) harboring pUA66-lacZ and the indicated plasmid. Cells were grown for ~3-4 hours in M9 minimal medium containing 0.2% casamino acids, 0.4% glycerol, 0.2% L-arabinose, and 0.1 mM IPTG to ABS_{600} ~0.2 prior to flow cytometry analysis. The reported values are the absolute fluorescence minus autofluorescence from cells lacking GFP. (**B**) Transformation efficiencies in the absence or presence of *cas3*. BW25113 Δ*cas3*::*cat* or NM500 *cas3*$^+$ cells were transformed with 50 ng of the indicated plasmid and plated on LB agar with ampicillin and kanamycin, and the number of colonies was counted. The differences in transformation efficiencies between strains may be attributed to switching cuvette manufacturers. (**C**) GFP repression following excision of the resistance cassette or in the presence of *cas3*. BW25113 Δ*cas3* or NM500 *cas3*$^+$ cells harboring pUA66-lacZ and pCRISPR.ind, pCRISPR.ind-T2 (T2), pCRISPR.ind-NT2 (NT2), or pCRISPR.ind-mviM (*mviM*) were grown as indicated in **A**. Repression is calculated as the ratio of the autofluorescence-subtracted fluorescence for pcrRNA.ind and each single-spacer plasmid. See Figure 2 for more information. Values represent the geometric mean and S.E.M. from independent experiments starting with three separate colonies.



Supplementary Figure S5. Extended information for the growth assays. (**A**) Individual ABS_{600} values for the growth assays. Raw data values are reported. The data represent those shown in Figure 4D with the exception of the last column showing growth in media containing four sugars. Dots represent individual measurements from independent cultures. (**B**) Doubling times of MG1655 Δ*cas3*::*cat* cells harboring the constitutive pcrRNA.con plasmid grown in minimal medium with the indicated sugar as the sole carbon source. Values represent the geometric mean and S.E.M. from independent experiments starting with three separate colonies.

SUPPLEMENTARY TABLES

Supplementary Table S1. Strains used in this work.

Strains	Genotype	Source	Stock #
BW25113	*Escherichia coli* K12 F^- DE(*araD-araB*)567 *lacZ*4787(del)(::*rrnB*-3) λ^- *rph*-1 DE(*rhaD-rhaB*)568 *hsdR*514	CGSC [a] #7636	pCB294
BW25113 Δ*cas3*::*cat*	BW25113 [Δ*cas3* P_{cse1}]::[*cat* P_{J23119}]	This study	pCB385
BW25113 Δ*cas3*	BW25113 [Δ*cas3* P_{cse1}]::[P_{J23119}]	This study	pCB400
BW25113 ΔCRISPR-Cas	BW25113 [Δ*cas3-cse1-cse2-cas7-cas5-cas6e*-CRISPR1]::*cat*	This study	pCB401
NM500 *cas3*$^+$	NM500 [ΔP_{cse1}]::[*cat* P_{J23119}]	This study	pCB402
MG1655	*Escherichia coli* K-12 F^- λ^- *ilvG*$^-$ *rfb*-50 *rph*-1	Storz lab (NIH)	pCB1
MG1655 Δ*cas3*::*cat*	MG1655 [Δ*cas3* P_{cse1}]::[*cat* P_{J23119}]	This study	pCB386

[a] CGSC: Coli genetic stock center (http://cgsc.biology.yale.edu).

Supplementary Table S2. Plasmids used in this work.

Plasmid	Description	Resistance marker	Source	Stock #
pUA66-lacZ	*lacZ* promoter upstream of GFP	Kanamycin	OpenBiosystems	pCB338
pUA66-araB	*araB* promoter upstream of GFP	Kanamycin	OpenBiosystems	pCB208
pUA66-xylA	*xylA* promoter upstream of GFP	Kanamycin	Ref. (3)	pCB289
pUA66-rhaB	*rhaB* promoter upstream of GFP	Kanamycin	Ref. (3)	pCB292
pBAD18	L-arabinose-inducible plasmid with *araC* regulator	Ampicillin	Ref. (3)	pCB284
pcrRNA.ind	pBAD18 with single repeat	Ampicillin	This study	pCB359
pcrRNA.ind-T1	pcrRNA.ind with spacer T1	Ampicillin	This study	pCB360
pcrRNA.ind-T2	pcrRNA.ind with spacer T2	Ampicillin	This study	pCB361
pcrRNA.ind-T3	pcrRNA.ind with spacer T3	Ampicillin	This study	pCB362
pcrRNA.ind-T4	pcrRNA.ind with spacer T4	Ampicillin	This study	pCB363
pcrRNA.ind-T5	pcrRNA.ind with spacer T5	Ampicillin	This study	pCB364
pcrRNA.ind-T6	pcrRNA.ind with spacer T6	Ampicillin	This study	pCB365
pcrRNa.ind-NT1	pcrRNA.ind with spacer NT1	Ampicillin	This study	pCB366
pcrRNA.ind-NT2	pcrRNA.ind with spacer NT2	Ampicillin	This study	pCB367
pcrRNA.ind-NT3	pcrRNA.ind with spacer NT3	Ampicillin	This study	pCB368
pcrRNA.ind-NT4	pcrRNA.ind with spacer NT4	Ampicillin	This study	pCB369

Supplementary Table S2. Continued.

Plasmid	Description	Resistance marker	Source	Stock #
pcrRNA.ind-NT5	pcrRNA.ind with spacer NT5	Ampicillin	This study	pCB370
pcrRNA.ind-NT6	pcrRNA.ind with spacer NT6	Ampicillin	This study	pCB371
pcrRNA.ind-LM	pcrRNA.ind with spacers T2-*mviM*	Ampicillin	This study	pCB372
pcrRNA.ind-LMM	pcrRNA.ind with spacers T2-*mviM*-*mviM*	Ampicillin	This study	pCB373
pcrRNA.ind-LMMM	pcrRNA.ind with spacers T2-*mviM*-*mviM*-*mviM*	Ampicillin	This study	pCB374
pcrRNA.ind-MLMM	pcrRNA.ind with spacers *mviM*-T2-*mviM*-*mviM*	Ampicillin	This study	pCB375
pcrRNA.ind-MMLM	pcrRNA.ind with spacers *mviM*-*mviM*-T2-*mviM*	Ampicillin	This study	pCB376
pcrRNA.ind-MMML	pcrRNA.ind with spacers *mviM*-*mviM*-*mviM*-T2	Ampicillin	This study	pCB377
pcrRNA.ind-MMMM	pcrRNA.ind with spacers *mviM*-*mviM*-*mviM*-*mviM*	Ampicillin	This study	pCB378
pcrRNA.ind-LLLL	pcrRNA.ind with spacers T2-T2-T2-T2	Ampicillin	This study	pCB403
pcrRNA.con	pcrRNA.con with synthetic constitutive promoter	Ampicillin	This study	pCB379
pcrRNA.con-lacZ	pcrRNA.con with spacer T2	Ampicillin	This study	pCB380
pcrRNA.con-araB	pcrRNA.con with spacer *araB*	Ampicillin	This study	pCB381
pcrRNA.con-xylA	pcrRNA.con with spacer *xylA*	Ampicillin	This study	pCB382
pcrRNA.con-rhaB	pcrRNA.con with spacer *rhaB*	Ampicillin	This study	pCB383
pcrRNA.con-araB/rhaB/xylA/T2	pcrRNA.con with spacers *araB*-*rhaB*-*xylA*-T2	Ampicillin	This study	pCB384

Supplementary Table S3. Oligonucleotides used in this work.

Name	Sequence
J23119-pKD3.for	GCTAGCATTATACCTAGGACTGAGCTAGCTGTCAATCCATATGAATATCCTCCTTAG
J23119-pKD3.rev	TGTAGGCTGGAGCTGCTT
HR-cas3.for	TACAATTAACCTATACATATATTAAGATGTGTTGAATTGTGCTAGCATTATACCTAGGAC
HR-cas3.rev	TGATATCATCGATAATACTAAAAAAACAGGGAGGCTATTATGTAGGCTGGAGCTGCTT
HR-CRISPR.for	ACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATTCCATATGAATATCCTCCTTAG
HR-casA.rev	CTTTTAATTTCCCGGTATGAGATTTTATATTCACAGTATGTGTAGGCTGGAGCTGCTT
pcrRNA.ind.for	CCACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGAAAAAAAAACCCCGCCCCTGACAGG GCGGGGTTTTTTTTA
pcrRNA.ind.rev	AAGCTTAAAAAAAACCCCGCCCTGTCAGGGGCGGGGTTTTTTTTTCGGTTTATCCCCGCTG GCGCGGGGAACTCGAGGTGGTACC
pcrRNA.con.for	TTTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGCG
pcrRNA.con.rev	CTAGCGCTAGCATTATACCTAGGACTGAGCTAGCTGTCAAATGCA
T2.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTACACTTTATGCTTCCGGCTCGT ATGT
T2.rev	TCGAACATACGAGCCGGAAGCATAAAGTGTAAAGCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
NT2.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCATAAAGTGTAAAGCCTGGGGTGCCT AATG
NT2.rev	TCGACATTAGGCACCCCAGGCTTTACACTTTATGCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
T3.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGAAACAGCTATGACCATGATTACGGAT TCAC
T3.rev	TCGAGTGAATCCGTAATCATGGTCATAGCTGTTTCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC
NT3.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGCGATTAAGTTGGGTAACGCCAGGGT TTTC
NT3.rev	TCGAGAAAACCCTGGCGTTACCCAACTTAATCGCCGGTTTATCCCCGCTGGCGCGGGGAAC TCGAGGTGGTAC

Supplementary Table S3. Continued.

Name	Sequence
T1.fwd	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGCCCTTTCGTCTTCACACTCGAGCACGACAG
T1.rev	TCGACTGTCGTGCTCGAGTGTGAAGACGAAAGGGCGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
T4.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGAGATATACATATGAGTAAAGGAGAAGAACT
T4.rev	TCGAAGTTCTTCTCCTTTACTCATATGTATATCTCGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
T5.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGTGATGCAACATACGGAAAACTTACCCTTAA
T5.rev	TCGATTAAGGGTAAGTTTTCCGTATGTTGCATCACGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
T6.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGTGATACCCTTGTTAATAGAATCGAGTTAAA
T6.rev	TCGATTTAACTCGATTCTATTAACAAGGGTATCACGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
NT1.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGACGAAAGGGCCTCGTGATACGCCTATTTTT
NT1.rev	TCGAAAAAATAGGCGTATCACGAGGCCCTTTCGTCGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
NT4.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTCTTCTCCTTTACTCATATGTATATCTCC
NT4.rev	TCGAGGAGATATACATATGAGTAAAGGAGAAGAACGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
NT5.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGTAAGTTTTCCGTATGTTGCATCACCTTCA
NT5.rev	TCGATGAAGGTGATGCAACATACGGAAAACTTACCGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
NT6.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGTATCACCTTCAAACTTGACTTCAGCACGT
NT6.rev	TCGAACGTGCTGAAGTCAAGTTTGAAGGTGATACCGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC

Supplementary Table S3. Continued.

Name	Sequence
araB.fwd	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGATTAGCGGATCCTACCTGACGCTTTTTATC
araB.rev	TCGAGATAAAAAGCGTCAGGTAGGATCCGCTAATCGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
xylA.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGAGTGCCCAATATTACGACATCATCCATCA
xylA.rev	TCGATGATGGATGATGTCGTAATATTGGGCACTCCGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
rhaB.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGGTCGCGAATTCAGGCGCTTTTTAGACTGGT
rhaB.rev	TCGAACCAGTCTAAAAAGCGCCTGAATTCGCGACCGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
mviM.for	CACCTCGAGTTCCCCGCGCCAGCGGGGATAAACCGAGCGCGGGCAGGGTATTCTCATCAAACCCA
mviM.rev	TCGATGGGTTTGATGAGAATACCCTGCCCGCGCTCGGTTTATCCCCGCTGGCGCGGGGAACTCGAGGTGGTAC
lacZ-qPCR.fwd	CGGCGTATCGCCAAAATCAC
lacZ-qPCR.rev	ATGGGTAACAGTCTTGGCGG
araB-qPCR.fwd	TACCAGTGCGTTAGGCTGTG
araB-qPCR.rev	CTGGACCCGATCCTCAATCG
xylA-qPCR.fwd	CGCCCCACAGGACATAGTTT
xylA-qPCR.rev	GGAACGGCCAACTGCTTTAC
rhaB-qPCR.fwd	TCACTTTCCGGGATCGGTTG
rhaB-qPCR.rev	TTCAGCGAGTGCTTCAGGAG

Supplementary Table S4. Protospacers in this work.

Spacer name	Target strand[a]	Distance from TSS[b]	Protospacer sequence[c]
T1	T	-141	**AGG**CCCTTTCGTCTTCACaCTCGAGCACGACAG
T2/lacZ	T	-37	**AGG**CTTTACACTTTATGCTTCCGGCTCGTATGT
T3	T	+27	**AGG**AAACAGCTATGACCATGATTACGGATTCAC
T4	T	+149	**AGG**AGATATACATATGAGTAAAGGAGAAGAACT
T5	T	+263	**AGG**TGATGCAACATACGGAAAACTTACCCTTAA
T6	T	+506	**AGG**TGATACCCTTGTTAATAGAATCGAGTTAAA
NT1	N	-129	**AAG**ACGAAAGGGCCTCGTGATACGCCTATTTTT
NT2	N	-20	**AAG**CATAAAGTGTAAAGCCTGGGGTGCCTAATG
NT3	N	+119	**AAG**GCGATTAAGTTGGGTAACGCCAGGGTTTTC
NT4	N	+180	**AAG**TTCTTCTCCTTTACTCATATGTATATCTCC
NT5	N	+290	**AGG**GTAAGTTTTCCGTATGTTGCATCACCTTCA
NT6	N	+515	**AGG**GTATCACCTTCAAACTTGACTTCAGCACGT
araB	T	-53	**AAG**ATTAGCGGATCCTACCTGACGCTTTTTATC
xylA	T	-19	**AGG**GAGTGCCCAATATTACGACATCATCCATCA
rhaB	T	-33	**AAG**GTCGCGAATTCAGGCGCTTTTTAGACTGGT
mviM[d]	N/A	N/A	**AAG**AGCGCGGGCAGGGTATTCTCATCAAACCCA

a) Characteristics of the target strand, which is complementary to the spacer: T, template strand of gene; N, non-template strand of the gene.
b) Distance from the transcriptional start site (TSS) to the closest end of the PAM. Negative and positive values are upstream and downstream of the TSS, respectively.
c) PAMs are in bold red lettering. CRISPR spacers were designed to match the protospacer sequence.
d) Targets a protospacer in *Salmonella typhimurium* LT2 and has been shown to be non-targeting in *E. coli*(4).

Supplementary Table S5. Promoter sequences.

Promoter	Sequence[a]
lacZ	CTTTCGTCTTCACACTCGAGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGC GCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTA TGCTTCCGGCTCGTATGTTGTGTGG**A**ATTGTGAGCGGATAACAATTTCACACAGGAA ACAGCTATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGG GAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACAGGATCCTCTAGATTTA AGAA
araB	CCTGTCTCTTGATCAGATCTGGCCTCAATCGGCGTTAAACCCGCCACCAGATGGGCG TTAAACGAGTATCCCGGCAGCAGGGGATCATTTTGCGCTTCAGCCATACTTTTCATA CTCCCACCATTCAGAGAAGAAACCAATTGTCCATATTGCATCAGACATTGCCGTCAC TGCGTCTTTTACTGGCTCTTCTCGCTAACCCAACCGGTAACCCCGCTTATTAAAAGC ATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTGTCTAT AATCACGGCAGAAAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCC ATAGCATTTTTATCCATAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTC TCTACTGTTTCTCCAT**A**CCCGTTTTTTTGGATGGAGTGAAACGATGGCGATTGCAAT TGGCCTCGATTTTGGCAGTGATTCTGTGCGAGCTTTGGCGGTGGACTGCGCTACCGG TGAAGCTCGAGGGGATCCTCTAGA
xylA	CGAGGCCCTTTCGTCTTCACGGTGTAGGGCCTTCTGTAGTTAGAGGACAGTTTTAAT AAGTAACAATCACCGCGATAAACGTAACCAATTTTTAGCAACTAAACAGGGGAAAAC AATTACAGATTTTTATCTTTCGATTACGATTTTTGGTTTATTTCTTGATTTATGACC GAGATCTTACTTTTGTTGCGCAATTGTACTTATTGCATTTTTCTCTTCGAGGAATTA CCCAGTTTCATCATTCCATTTTATTTTGCGAGCGAGCGCACACTTGTGAATTATCTC AATAGCAGTGTGAAATAACATAATTGAGCAACTGAAAGGGAGTGCCCAATATTACGA C**A**TCATCCATCACCCGCGGCATTACCTGATTATGGAGTTCAATATGCAAGCCTATTT TGACCAGCTCGATCGCGTTCGTTATGAAGGCTCAAAATCCTCAAACCCGTTAGCATT CCGTCACTACAATCCCGACGAACTGGTGTTGGGTAAGCGTATGTAATCTAGATTTAA GAAGGAGAT
rhaB	CCTGTCTCTTGATCAGATCTGTTCTATCGCCACGGACGCGTTACCAGACGGAAAAAA ATCCACACTATGTAATACGGTCATACTGGCCTCCTGATGTCGTCAACACGGCGAAAT AGTAATCACGAGGTCAGGTTCTTACCTTAAATTTTCGACGGAAAACCACGTAAAAAA CGTCGATTTTTCAAGATACAGCGTGAATTTTCAGGAAATGCGGTGAGCATCACATCA CCACAATTCAGCAAATTGTGAACATCATCACGTTCATCTTTCCCTGGTTGCCAATGG CCCATTTTCCTGTCAGTAACGAGAAGGTCGCGAATTCAGGCGCTTTTTAGACTGGTC GT**A**ATGAAATTCAGCAGGATCACATTATGACCTTTCGCAATTGTGTCGCCGTCGATC TCGGCGCATCCAGTGGGCGCGTGATGCTGGCGCGTTACGAGCGTGAATGGGATCCTC TAGATTTAAGAA

a) Sequences highlighted in gray are from pUA66, indicating where each promoter was inserted into the plasmid. The underlined and bolded base is the previously mapped transcriptional start site.

SUPPLEMENTARY REFERENCES

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